REMARKS

Claims 1 through 11 are pending in the application.

Priority

The examiner states that the instant claims 1 and 6 are awarded priority back to January 19, 2001, which is the 371 date of the prior application. It is believed that examiner meant to refer to the filing date of the instant continuation-in-part application filed 8/27/2002.

Specification

The specification has been amended to include sequence identifiers SEQ ID NO:1 and SEQ ID NO:2. However the spelling of "Neisseria gonorrhoeae" has not been amended since this is the spelling used by **CDC - Centers for Disease Control and Prevention, Department of Health and Human Services** (see enclosed CDC web site print-out). Therefore, the spelling is presumed to be correct and has not been changed.

Claim Objection

The claims 1 and 6 have been amended to include the proper sequence identifiers.

REJECTIONS

Claim Rejections - 35 U.S.C. 112

Claims 1-11 stand rejected under 35 U.S.C. 112, 2nd paragraph, as being indefinite. The examiner refers to the wording "undergoing" and to the spelling of "Neisseria gonorrhoeae".

In regard to the wording "undergoing at least one reaction of ", Applicant would like to point out that the phrase "undergoing at least one reaction" is the lead-in for two specific reactions that can take place alternatively or together (therefore: at least one reaction of). The two reactions are **binding** and **being endocyted**. The claim language when concentrating on the two actions reads as follows:

*a protein undergoing at least one reaction of **binding** to a receptor ..... and of **being endocyted***

As pointed out above the spelling of "Neisseria gonorrhoeae" is used by the CDC. The spelling is therefore presumed to be correct and has not been changed.

Rejection under 35 U.S.C. 103

Claims 1-4, 6-8 and 10-11 stand rejected under 35 U.S.C. 103(a) as being unpatentable over *Slater et al. (J. of Immunology, Vol. 140, 807-811, No. 3, 1988)* and *Borodic (US 6,429,189)*.

The object to be solved by the present invention is to provide a compound capable of suppressing anti-allergic reactions by inhibiting the secretion of endogenous vasoconstrictive amines from mast cells and/or basophils for more than only few days. The invention has solved this object by providing the hybrid proteins as defined in claims 1 and 6, respectively. The hybrid protein of the present invention employs **neurotoxins** and prevents allergic reactions by inhibiting the secretion of endogenous vasoconstrictive amines stored in mast cells that are the effector cells for allergic reactions and secrete histamine and other effector molecules after stimulation with allergens. The hybrid protein described and claimed in the present application is able to inhibit the secretion process and therefore to prevent allergic reactions for a period of 2 - 3 months **without killing the cells.**

The examiner's position is that *Slater et al.* disclose immunotoxins/toxins covalently conjugated to specific antibodies and also disclose the avid binding of IgE antibodies to FcR on mast cells and basophils therefore suggesting the possible use of IgE immunotoxins in the treatment of malignant mastocytosis. According to the Examiner, this reference also discloses that IgE-ricin A chain significantly reduces the cutaneous histamine content and, moreover, discloses immunotoxins to bind to surface antigens through the binding side on the Fab portion of the molecule. The examiner cites the secondary reference to *Borodic* in order to show clostridium botulinum as directly or indirectly influencing the system involving mast cells, histamine, serotonin such that blocked physiological response (mast cell secretion or degranulation) results. According to the examiner, the secondary reference teaches inhibition of secretion when mast cells are pretreated with botulinum toxin and a derivative strain that has produced a non-neurotoxin protein (botulinum C2) demonstrating selective interaction with mast cells. Moreover, the examiner points out that it is general knowledge in the art that botulinum

toxin and tetanus toxin are produced by closely related microorganisms and are structurally and functionally similar. Therefore, examiner argues that it would have been obvious to combine the teachings of the two prior art references because *Slater et al.* teaches a conjugate having affinity to mast cells and basophils and causing depletion of histamine levels. Even though the primary reference does not specifically teach botulinum or tetanus toxin, the secondary reference teaches the use of such toxins for inhibiting secretion in mast cells.

The hybrid protein of the present invention differs fundamentally from **immunotoxins** and related proteins which are well known in the art, e.g., in the field of tumor therapy. These prior art fusion proteins consist of a cell binding element (e.g., an antibody or antibody fragment) and a **cytotoxic element**. The cytotoxic element may be, e.g., a subunit of ricin, as disclosed by *Slater et al.*

The **botulinum neurotoxins** and the **tetanus toxin** are completely different from the **cytotoxins** described in *Slater et al.*

1. The subunit of ricin (chain A of ricin) as described by *Slater et al.* is not a protease; it is an N-glucosidase.
2. The ricin A chain is active as a **cytotoxic** agent in any type of cell because it blocks an essential process (protein biosynthesis) necessary for the cell's survival. The botulinum neurotoxins and the tetanus toxin are only **active in cholinergic nerve cells** - no cells other than cholinergic nerve cells are affected by the neurotoxins proposed by the present invention because the **neurotoxins cannot enter other cells**. The botulinum neurotoxins and the tetanus toxin act in a highly specific manner on the signal transduction from nerve cells to the muscle. They cleave proteins that are essential for the secretion of acetylcholine, the so-called SNARE proteins.

Based on this knowledge, *Slater et al.* propose a logical approach: combining the unspecific cytotoxic activity with a cell-specific anchor - a concept which is generally popular in tumor therapy. Such a fusion protein enters the cell and destroys it. The disadvantage brought about by such agents is that the dying cell releases its contents, and

this may cause adverse reactions. Moreover, the number of cells is controlled by feedback mechanisms so that the decrease in cell numbers will cause increased generation of new cells that must also be deactivated. This feedback control requires a continuos injection of the toxin-IgE fusion protein. Additionally, the cytotoxic element, i.e., the ricin A chain according to *Slater et al.*, inhibits protein synthesis and consequently kills the cell.

In contrast thereto, the hybrid protein according to the invention containing the light chain of a botulinum neurotoxin, the light chain of the tetanus toxin, or a catalytically active fragment of the botulinum or tetanus neurotoxin light chain as defined in claim 1 **does not kill the cell**. Rather, it **inhibits the cell's secretion mechanism** - but vital functions are not influenced and the cell stays alive.

The cell treated with the hybrid protein of the present invention is only disabled in regard to secretion of those molecules that are responsible for the allergic reaction. Because it is known in regard to nerve cells that the secretion mechanism of the nerve cells is inactivated for about 3 months, an allergic reaction will be prevented for this time period.

This is certainly a tremendous difference in comparison to the situation that occurs when mast cells are destroyed as disclosed in *Slater et al.* The mast cells are replaced after a few days. When cells are replaced, the new cells, however, are able to react in response to an allergen and thus cause the organism to again react allergically. Thus, the anti-allergic effect provided by the compounds of *Slater et al.* would last only for a very short period of time.

Moreover, it cannot be ruled out that the cells killed by the toxic peptide/protein will release their stored amines in the course of dying; this would lead to an acute allergic response.

Thus, the subject matter of the present claims is distinct from what is disclosed in *Slater et al.* The Examiner therefore properly did not raise the issue of novelty but applied the secondary reference to *Borodic* in order to show the use of botulinum toxin in connection with inhibiting secretion in mast cells.

Applicant would like to emphasize that seven different botulinum neurotoxins (that is, of type A, B, C1, D, E, F, and G) have been described previously (e.g., Bigalke, H.

(1999), Ref. 3 in the instant specification). These botulinum neurotoxins consist of a heavy chain (molecular weight of approximately 100 kDa) and a light chain (molecular weight of 50 kDa). The heavy chain is responsible for binding to the cholinergic nerve cell and for translocation through the nerve cell membrane. The light chain is a protease which can not penetrate membranes in its purified form.

In contrast to the above neurotoxins, botulinum toxin type C2, the one that is disclosed in *Borodic*, is a **cytotoxin** with ADP-ribosylating activity. Botulinum toxin type C2 acts by ADP-ribosylation of actin monomers. This cytotoxin kills cells non-specifically, inclusive of non-neuronal cells.

The Examiner has not made a distinction between the botulinum neurotoxins A, B, C1, D, E, F, and G, on the one hand, and botulinum toxin C2, on the other hand. Thus, she concluded that the secondary reference *Borodic* in combination with *Slater et al.* teaches the invention as claimed. This is not so.

Borodic discloses the use of a cytotoxin (non-neurotoxin!) for the treatment of headaches and other pains. The Abstract of *Borodic* reads as follows:

> *"Pharmaceutical applications of a chemodenervating agent reduce pain by altering release of pain and inflammation-mediating autocoids, with a duration of action between 12-24 weeks. The limiting factor in dosing for this application is weakness and paralysis created by higher doses of the chemodenervating pharmaceutical. This weakness and paralysis is mediated by action of the* ***neurotoxin component*** *of the chemodenervating pharmaceutical. The invention described herein represents a novel mechanism and pharmaceutical formulation which* ***eliminates the neurotoxin component*** *of the chemodenervating pharmaceutical,* ***while retaining the cytotoxin component*** *which provides an essential bioeffect for the relief of pain and inflammation. The invention allows for improvement in administering the pharmaceutical agent for the reduction of pain and/or inflammation without causing muscular weakness and paralysis."* (emphasis added)

In other words, the botulinum **neurotoxin** responsible for muscular weakness and paralysis according to *Borodic* is no longer a component of the pharmaceutical formulation provided. The formulation comprises instead botulinum cytotoxin C2 but no neurotoxin (see Borodic, col. 5, lines 17-27; col. 5, line 67, to col. 6, line 3; col. 6, lines 35-57; col. 7, lines 4-26). Claim 1 of *Borodic* states explicitly that the purified protein derived from *Clostridium botulinum* has no neurotoxin properties. As specified in claims 2 and 3, the protein is of the type C2.

Thus, although *Borodic* discusses the secretion of histamine as a possible cause of headaches, the patent document does not provide a skilled person in the art with the suggestion to replace the ricin subunit disclosed in *Slater et al.* with the light chain of a botulinum **neurotoxin**, with the **light chain of the tetanus toxin**, or with a proteolytically active fragment of the **botulinum or tetanus neurotoxin light chain** as defined in claims 1 and 6. It teaches the use of a cytotoxin and the elimination of neurotoxins.

It was not known in the art prior at the time of the present invention that the substrates of the botulinum neurotoxins and of the tetanus toxin are also present in mast cells and that they are involved in the release of histamine from these cells. It was therefore not obvious to employ a part of the botulinum/tetanus neurotoxin that, physiologically, was only known to be active in cholinergic nerve cells and supposed to be inactive in regard to other cells. It was thus not obvious for a person of ordinary skill in the art to use a toxin which is active on synaptosomal proteins (i.e. proteins from nerve cells) in a very specialized, non-neuronal cell population (mast cells), because it was not known at the time of the invention how mast cells deliver histamine and other amines. The use of neurotoxins for inhibiting the degranulation of mast cells is an entirely novel concept.

The publication of *Slater et al.* describes that mast cells can be **killed** by a cytotoxin (ricin) fusion protein. Therefore, it is an inventive step to synthesize a hybrid protein comprising a component that is physiologically active in nerve cells for the purpose of treating a completely different type of cell and to do so without killing the cell!

A further advantage of the present invention is the **absence of dying cells**, because the employed neurotoxins are not cytotoxic. Therefore, a temporary increase in

histamine levels is avoided. It is a completely new approach in anti-allergic therapy to avoid the destruction of mast cells by cytotoxins like ricin and, instead, to keep them alive and simply inactivate the secretion machinery. This new approach leads to a longer-lasting therapeutic effect, because the number of mast cells is not decreased and resynthesis of cells will not take place.

In summarizing the above: at the time of the invention, it was not known that the botulinum neurotoxins and the tetanus toxin would be able to exert their bioactivity on cells other than neuronal cells. The important contribution of the present invention resides in proposing hybrid proteins comprising:

(i) a proteolytically active portion disrupting the allergic reaction of a mast cell or basophil **without killing the cell**, and

(ii) a targeting moiety directing the proteolytically active portion (i) to the desired cell type (basophil, mast cell).

The invention surprisingly found that **neurotoxins** such as the light chain of botulinum **neurotoxin** type A, B, C1, D, E, F, and G was also active in mast cells, a cell type completely different from nerve cells, once the light chain was introduced into the mast cell by using a specific anchor sequence derived from, e.g., immunoglobulin type E (IgE). In this context, it is noteworthy that mast cells do not originate from neuronal stem cells and that they are different from nerve cells.

Consequently, it was surprising that the hybrid proteins as defined in the claims are suitable agents for suppressing allergic reactions elicited by the secretion of, e.g., histamine, from mast cells and basophils.

Claims 1 through 11 are therefore believed to be allowable.

CONCLUSION

In view of the foregoing, it is submitted that this application is now in condition for allowance and such allowance is respectfully solicited.

Should the Examiner have any further objections or suggestions, the undersigned would appreciate a phone call or e-mail from the examiner to discuss appropriate amendments to place the application into condition for allowance.

Authorization is herewith given to charge any fees or any shortages in any fees

required during prosecution of this application and not paid by other means to Patent and Trademark Office deposit account 50-1199.

Respectfully submitted on March 5, 2004,

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GEH
Encl.: print-out CDC Neisseria gonorrhoeae (2 sheets)

Division of AIDS, STD, and TB Laboratory Research

Alert:
Fluoroquinolone Resistance

Gonorrhea Definitions:
Clinical laboratory
Surveillance definition

Antimicrobial Resistance
Surveillance: GISP
GISP protocol
Susceptibility Testing
Disk diffusion
Agar dilution (GISP)

Neisseria and Related Species
Identification
Natural Habitat
Pathogenicity
Prevalence
Taxonomy

Strain Typing
Auxotyping
Serotyping

Archives
CDC publications
Branch publications

Links: Other Resources

Gonorrhea, caused by Neisseria gonorrhoeae, is second only to chlamydial infections in the number of cases reported to the Centers for Disease Control and Prevention (CDC); 361,705 cases were reported to CDC in 2001 (2001 Sexually Transmitted Diseases Surveillance Report). The number of reported cases of gonorrhea increased steadily from 1964 to 1977, fluctuated through the early 1980s, increased until 1987, and since 1987 has decreased annually. The incidence of gonorrhea is highest in high-density urban areas among persons under 24 year of age who have multiple sex partners and engage in unprotected sexual intercourse. Increases in gonorrhea prevalence have been noted recently among men who have sex with men.

Antimicrobial resistance in N. gonorrhoeae contributed to the increase in cases of gonorrhea in the United States during the 1970s and 1980s. Antimicrobial resistance in N. gonorrhoeae occurs as plasmid-mediated resistance to penicillin and tetracycline, and chromosomally mediated resistance to penicillins, tetracyclines, spectinomycin, and recently to fluoroquinolones. The decline in gonorrhea prevalence may be attributed to recommendations by CDC (2002 Sexually Transmitted Diseases Treatment Guidelines) that only highly effective antimicrobial agents be used to treat gonorrhea.

The gonorrhea laboratory program at CDC includes efforts to:

a) ensure the accurate identification of N. gonorrhoeae;
b) determine the magnitude, nature, and diversity of antimicrobial resistance in N. gonorrhoeae; and
c) study the dynamics of gonococcal strain populations within communities.

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URL: http://www.cdc.gov/ncidod/dastlr/gcdir/gono.html
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